主　論　文　要　旨

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主 論 文 題 名

Intravital Two-photon Imaging of Ca^{2+} signaling in Secretory Organs of Yellow Cameleon Transgenic Mice
（イエローカメレオントランスジェニックマウスを用いた外分泌器官における生体内Ca2+イメージング）

（内 容 の 要 旨）

Intracellular Ca^{2+} signaling ($[Ca^{2+}]i$) is involved in almost all aspects of living process. The maintenance of intracellular Ca^{2+} concentration is under restrict regulation by keeping the Ca^{2+} gradient through activities of intracellular stores or membrane channels. $[Ca^{2+}]i$ elevation in acinar cells of secretory organs triggers fluid secretion, alteration of normal $[Ca^{2+}]i$ elevation may influence the secretory function of corresponding organs, or even cause pathological changes.

The establishment and evaluation of intravital $[Ca^{2+}]i$ visualization system is very important and practical in monitoring the $[Ca^{2+}]i$ dynamics in specific types of cells of secretory organs. By using this system, any alteration of the $[Ca^{2+}]i$ dynamics can be easily discovered and quantitatively assessed in living animals, which deepens the understanding of molecular mechanisms of related secretory diseases.

For establishing the system, a combination of Yellow Cameleon (YC) transgenic mice and two-photon microscope was used. The method fully takes advantage of the ratiometric and linear characteristics of genetically encoded Ca^{2+} indicator (GECI) YC3.60, which is composed of cyan fluorescent protein (CFP), calmodulin (CaM), a shorter linker peptide, the CaM-binding peptide of myosin light-chain kinase (M13) and yellow fluorescent protein (YFP). Upon binding of free Ca^{2+}, the Ca^{2+} responsive element calmodulin (CaM) of YC will change the efficiency of the two fluorescent protein CFP and YFP, thus a changing ratio, which is also called fluorescence resonance energy transfer (FRET) ratio, of the fluorescence density of YFP to CFP can be recorded and monitored.

For evaluation of the system, initially, three types of pharmacological agent were used to exam the physiological responses of secretory organs ex vivo. The different mechanisms of $[Ca^{2+}]i$ signaling were successfully confirmed in all the tested secretory organs (pancreas, salivary gland (SG), and lacrimal gland (LG)) of YC3.60 transgenic mice. Furthermore, intravital imaging of the secretory organs of living YC3.60 transgenic mice was examined to monitor the $[Ca^{2+}]i$ dynamics under cholinergic stimulations because autonomic nervous system regulates the normal physiological secretory functions. Increase of tear secretion and salivary secretion can be recorded spontaneously with intravital monitoring of the $[Ca^{2+}]i$ within LG and SG. The changes of ratiometric FRET ratio and real $[Ca^{2+}]i$ concentration can be calculated in the cell-specific monitoring, which will provide precise evidences to improve the understanding of secretory mechanisms in normal physiology. Finally, the intravital $[Ca^{2+}]i$ visualization system was used in model of postganglionic denervation of LG, a well-established experimental model of dry eye disease, to evaluate the $[Ca^{2+}]i$ changes in pathological state of LG. A marked attenuation of $[Ca^{2+}]i$ response to cholinergic stimulation was found in myoepithelial cells of LG.